5-1-1994

Exocrine Pancreatic Neoplasms in the Mummichog (Fundulus heteroclitus) from a Creosote-Contaminated Site

John W. Fournie

Wolfgang K. Vogelbein
Virginia Institute of Marine Science

Recommended Citation

Fournie, John W. and Vogelbein, Wolfgang K., Exocrine Pancreatic Neoplasms in the Mummichog (Fundulus heteroclitus) from a Creosote-Contaminated Site (1994). *Toxicological Pathology*, 22(3), 237-247.
doi: 10.1177/019262339402200302

TOXICOLOGIC PATHOLOGY ISSN:0192-6233

Volume 22, Number 3, 1994
Printed in U.S.A.

Exocrine Pancreatic Neoplasms in the Mummichog (*Fundulus heteroclitus*) from a Creosote-Contaminated Site*

JOHN W. FOURNIE[1] AND WOLFGANG K. VOGELBEIN[2]

[1]*U.S. Environmental Protection Agency, Center for Marine and Estuarine Disease Research, 1 Sabine Island Drive, Gulf Breeze, Florida 32561, and*
[2]*School of Marine Science, The College of William and Mary, Virginia Institute of Marine Science, Gloucester Point, Virginia 23062*

ABSTRACT

A high prevalence of exocrine pancreatic neoplasms occurred in mummichog, *Fundulus heteroclitus,* from a creosote-contaminated site in the Elizabeth River, Virginia. A total of 20 neoplasms were found in a group of about 1,300 fish obtained at this site over a 2-yr period. Of 240 fish collected during October 1991, 3.3% had pancreatic neoplasms. Adjusted total lesion prevalence for large adult fish (Size Class III: total length = 75–85 mm; Size Class IV: total length > 85 mm) was 6.7%. Pancreatic neoplasms were not observed in 234 fish collected at this site during May 1991, nor were they found in 420 fish obtained during fall 1991 from 1 uncontaminated and 6 moderately contaminated localities. Lesions involved both mesenteric and intrahepatic exocrine pancreas and ranged from well-differentiated acinar cell adenomas to poorly differentiated acinar cell carcinomas. One fish had an atypical acinar cell focus. All specimens with pancreatic neoplasms also had hepatocellular lesions. This epizootic of exocrine pancreatic neoplasia is the first to be reported in a wild fish population. Based on chemical characterization of the site and limited experimental data on chemically induced pancreatic carcinogenesis in other small fish species, the neoplasms were probably caused by exposure of the mummichog to chemical carcinogens in their environment.

Keywords. Exocrine pancreas; neoplasia; fish; pollution

INTRODUCTION

The number of reported neoplasm epizootics in fishes has increased in North America over the past 25 yr (13). Neoplasms affect nearly every cell and tissue type in fishes, and those involving the liver and epidermis have been associated with exposure to xenobiotic chemical contaminants. On the other hand, neoplasms of hemic, neural, connective tissue, and gonial origin are generally believed to be unrelated to environmental pollution (14). Several classes of chemical carcinogens have been shown to induce neoplasia in laboratory fishes (5, 18, 19) with polycyclic aromatic hydrocarbons (PAHs) thought to be responsible for many of the reported epizootics of liver cancer in wild fishes (e.g., 1, 26, 27, 33).

We report here the histology and prevalence of exocrine pancreatic neoplasms in mummichog, *Fundulus heteroclitus,* from a creosote-contaminated site in the Elizabeth River, Virginia. Previously, high prevalences of liver neoplasia have been reported in mummichog from that site (33). Sediments at this location contained exceptionally high concentrations (2,200 mg/kg dry sediment) of total PAHs believed to have originated from a nearby wood-treatment facility. This epizootic of exocrine pancreatic neoplasia is the first to be reported in a wild fish population.

MATERIALS AND METHODS

Mummichog with pancreatic neoplasms were part of approximately 1,300 fish collected between October 1989 and 1991. Fish were collected with baited minnow traps in a small tidal creek adjacent to a wood-treatment facility (Atlantic Wood Industries, Inc.) located on the Elizabeth River, in Portsmouth, Virginia. Fish were transported live to the laboratory and maintained in an open, circulating water system. Because these fish were used in a variety of unrelated studies, with some held for up to

* Address correspondence to: Dr. John W. Fournie, U.S. Environmental Protection Agency, Center for Marine and Estuarine Disease Research, 1 Sabine Island Drive, Gulf Breeze, Florida 32561.

0192-6233/94$3.00+$0.00

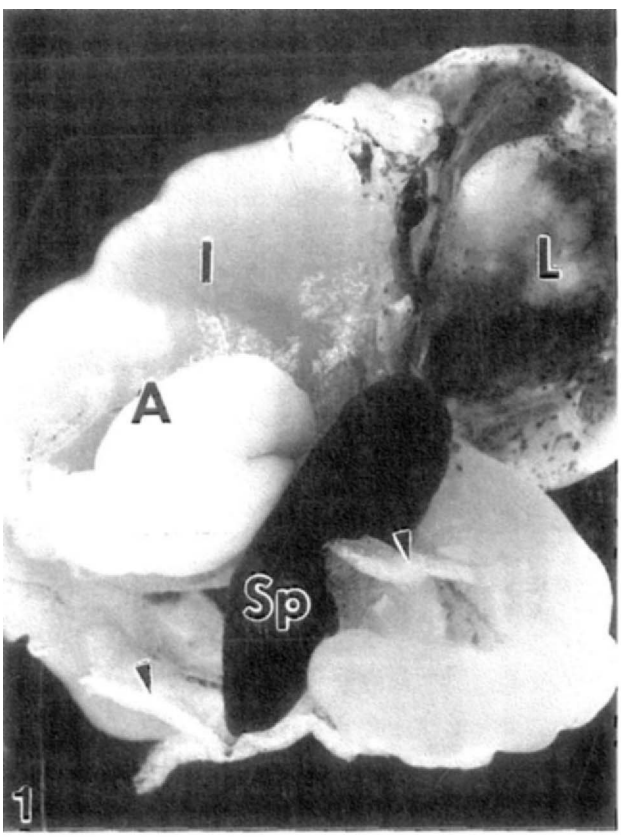
TABLE I.—Prevalences of exocrine pancreatic neoplasms in mummichog, *Fundulus heteroclitus*, from a creosote-contaminated site in the Elizabeth River, Virginia.

	Size Class			
Total length[a]	I ≤55	II 56–70	III 71–85	IV ≥86
May 1991 (N)	37	68	69	60
Adenoma	—	—	—	—
Carcinoma	—	—	—	—
October 1991 (N)	60	60	60	60
Adenoma	—	—	1 (1.7[b])	5 (8.3[b])
Carcinoma	—	—	2 (3.3[b])	—

[a] Total length in millimeters.
[b] Percentage of lesion prevalence in size class.

9 mo in the laboratory and others not examined for the presence of pancreatic neoplasms, they were not used to calculate lesion prevalences. Lesion prevalences reported here were obtained from a group of 474 mummichog collected during May and October 1991 and necropsied within 5 days after capture.

Mummichog were anesthetized in tricaine methanesulfonate, examined grossly, sexed, measured, and weighed. Visceral organs were dissected and examined. Tissue samples of liver, pancreas, and any grossly visible abnormal visceral masses were fixed in Bouin's solution for 48 hr and processed for routine paraffin histology. Sections were cut at



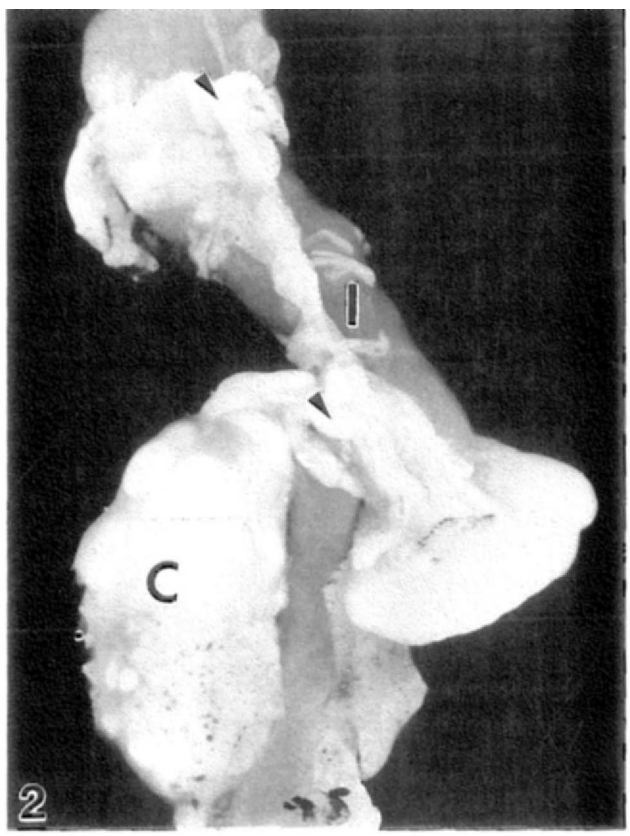


FIG. 1.—Visceral mass from mummichog (*Fundulus heteroclitus*) showing smooth, multinodular exocrine pancreatic adenoma (A). I = intestine; L = liver; Sp = spleen. Arrowheads indicate normal exocrine pancreas. ×6.15.

FIG. 2.—A large multilobulated acinar cell carcinoma (C) arising from the intestinal mesentery. I = intestine. Arrowheads indicate normal exocrine pancreas. ×6.1.

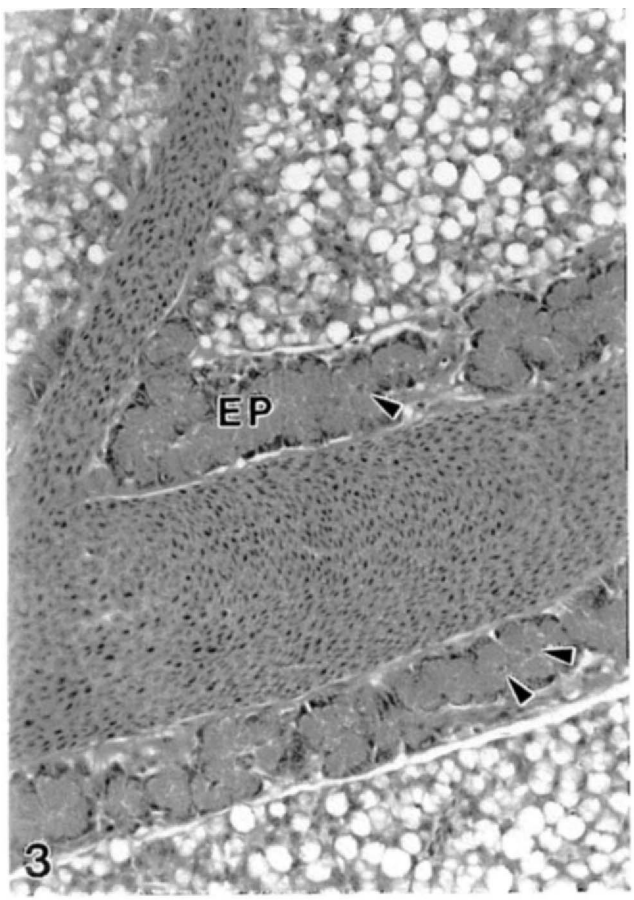
→

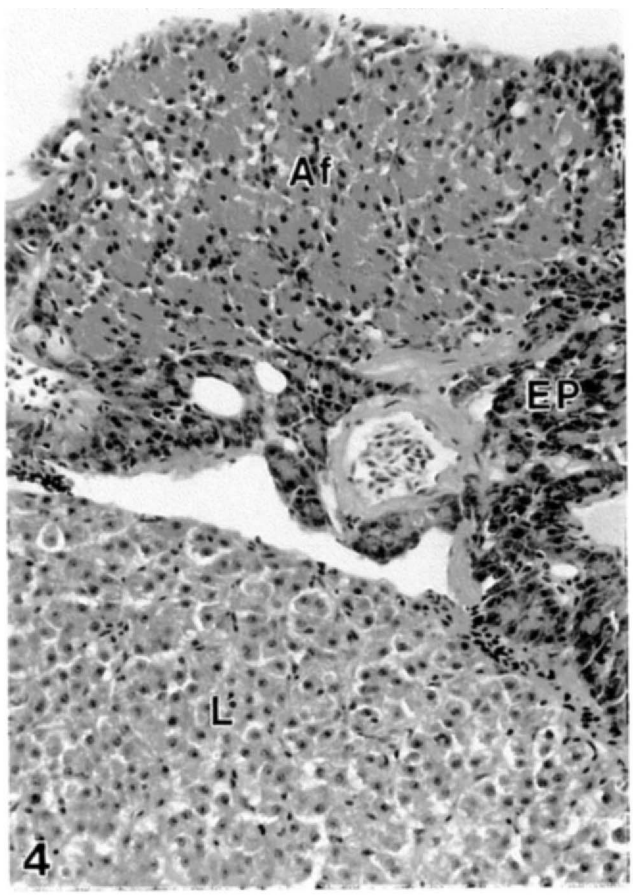
FIG. 3.—Light micrograph of normal, intrahepatic exocrine pancreas (EP) in the mummichog. Note acinar cells and nuclei of centroductular cells (arrowheads). H&E. ×244.

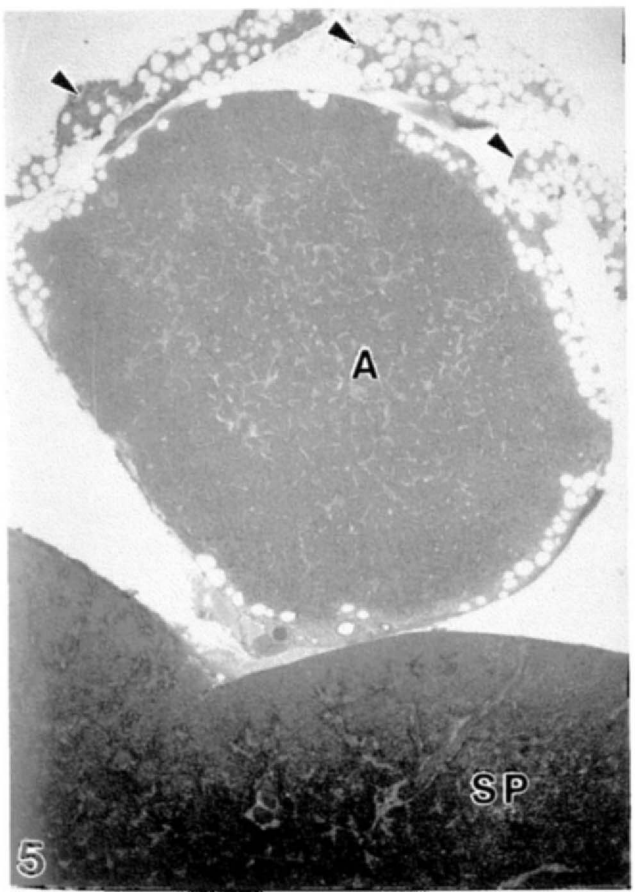
FIG. 4.—Acidophilic focus (Af) arising within mesenteric exocrine pancreas (EP) adjacent to liver (L). H&E. ×244.

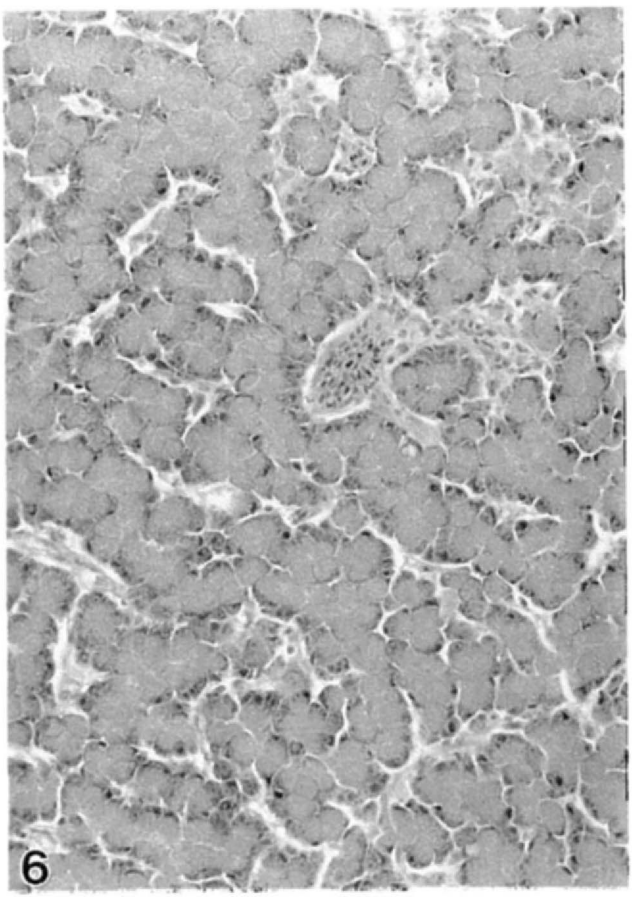
FIG. 5.—Low magnification of mummichog acinar cell adenoma (A) arising from mesenteric exocrine pancreas (arrowheads) at hilus of spleen (Sp). Note branching tubular arrangement of acinar cells and discrete lesion border. H&E. ×24.

FIG. 6.—Higher magnification of adenoma in Fig. 5 showing anastomosing, branched pancreatic tubules composed of well-differentiated acinar cells containing zymogen. H&E. ×244.









5 μm and stained with Harris' hematoxylin and eosin (25).

Selected tissues were also prepared for high-resolution light microscopy. Tissues were fixed for 2 hr in 1.25% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH = 7.4), dehydrated in a graded ethanol series, and embedded in glycol methacrylate (LKB Historesin). Blocks were sectioned on a Reichert 2050 Supercut microtome at 1–2 μm and stained with hematoxylin and eosin–phloxine.

A pancreatic neoplasm from a paraffin-embedded specimen was reclaimed and processed for transmission electron microscopy to verify the presence of zymogen granules. The tissue was cut out of the paraffin block with a razor blade, deparaffinized in xylene, rehydrated, osmicated, and processed by routine methods for electron microscopy (16). Ultra-thin sections were obtained with a diamond knife, stained with lead citrate and uranyl acetate, and examined in a Zeiss CEM 902 transmission electron microscope.

RESULTS

Lesion Prevalence

Twenty of approximately 1,300 mummichog collected from the creosote-contaminated site adjacent to Atlantic Wood Industries, Inc., exhibited exocrine pancreatic neoplasms. Pancreatic neoplasms were not observed in a total of 420 large adult fish (Size Classes III and IV) obtained from 7 other locations. Five of these localities were moderately PAH-contaminated sites in the Elizabeth River, 1 was a moderately PAH-contaminated site in the York River, Virginia, site, and 1 was a relatively uncontaminated site in the York River. Sixty fish comprised the sample from each site.

Lesion prevalences calculated from a subsample of 474 fish obtained in the spring and fall of 1991 are shown in Table I. Pancreatic neoplasms were not observed in 234 mummichog collected during May 1991, whereas 8 of the 240 fish captured during October 1991 were afflicted, resulting in an overall lesion prevalence of 3.3%. Pancreatic neoplasms were observed only in large adult fish. Three affected individuals belonged to Size Class III and 5 belonged to Size Class IV. Total length of fish with neoplasms ranged from 72 to 101 mm ($\bar{x}$ = 89.0 mm). Adjusted total lesion prevalence for fish belonging to Size Classes III and IV was 6.7%. Of these fish, 1.7% exhibited acinar cell carcinomas and 5.0% had acinar cell adenomas. A relationship between the occurrence of pancreatic neoplasms and gender was not apparent. Five of the 8 fish with lesions were males and 3 were females. Both individuals with acinar cell carcinomas were males. All specimens with pancreatic neoplasms also exhibited focal or neoplastic hepatocellular lesions.

Normal Morphology

Grossly, exocrine pancreas in the mummichog occurred as diffuse masses of acinar cells suspended in the visceral mesentery (Figs. 1 and 2), around the hepatic portal vasculature within the liver, and surrounding the gallbladder. Microscopically, exocrine pancreas was arranged as a continuous branching network of anastomosing tubules composed of acinar cells (Fig. 3). Although true terminal acini could not be demonstrated, some tubules ended in blind oval or round structures resembling the classical vertebrate acinus.

Acinar cells were pyramidal to cuboidal, had basally situated nuclei with prominent nucleoli, and contained numerous eosinophilic zymogen granules in the apical cytoplasm (Fig. 3). Basal portions of acinar cells stained basophilically and were devoid of zymogen granules. Centroductular cells were flattened epithelial cells with scant, pale-staining cytoplasm, located within the center of the pancreatic tubules (Fig. 3).

Acidophilic Focus

One fish had a small, well-demarcated lesion arising in the mesenteric pancreas that measured 245 μm in greatest diameter. Constituent acinar cells exhibited an increased zymogen content, reduced basal basophilia, and normal nuclear morphology (Fig. 4). This lesion was not observed in the spring and fall 1991 samples.

Acinar Cell Adenoma

Acinar cell adenomas occurred in the mesenteric pancreas of 10 specimens, in the exocrine pancreatic

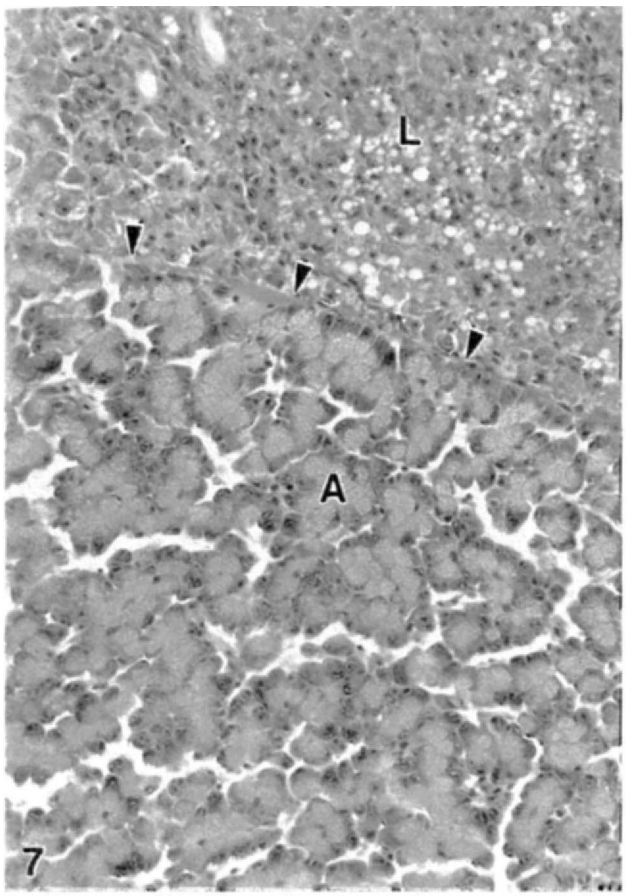
→

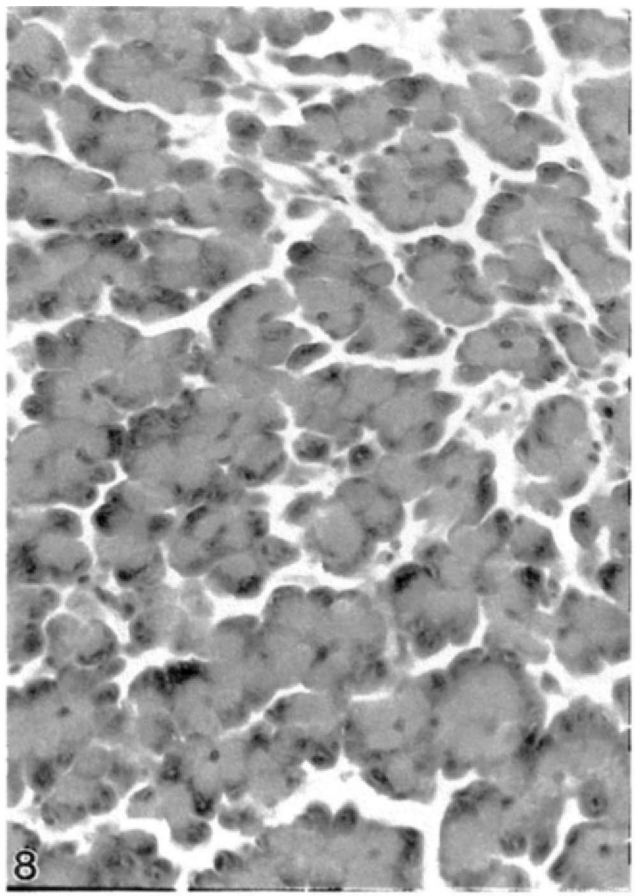
FIG. 7.—Adenoma (A) arising in the liver (L). Arrowheads indicate border between neoplasm and liver parenchyma. H&E. ×244.

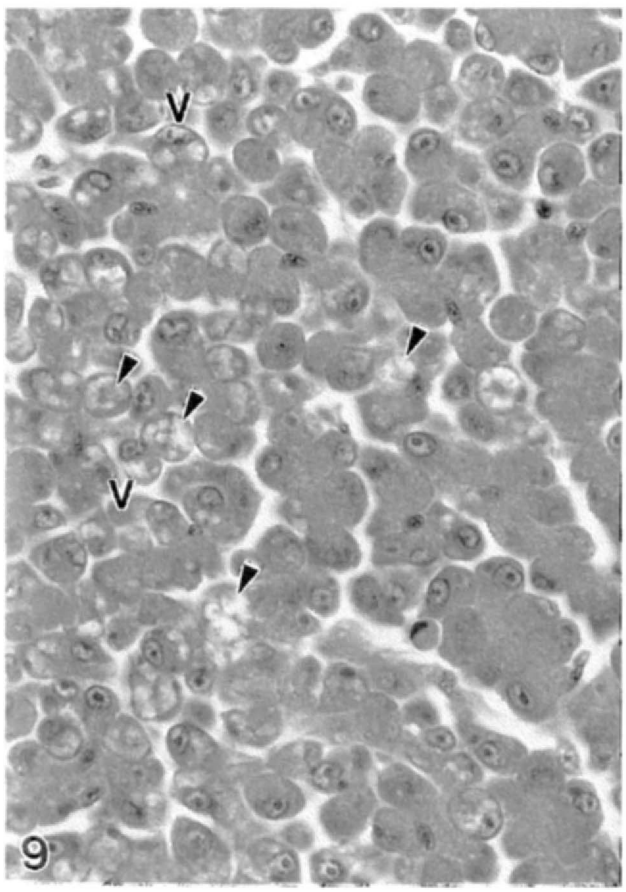
FIG. 8.—Adenoma showing acinar cells exhibiting atypical nuclei. Note elongated, flattened nuclei. H&E. ×390.

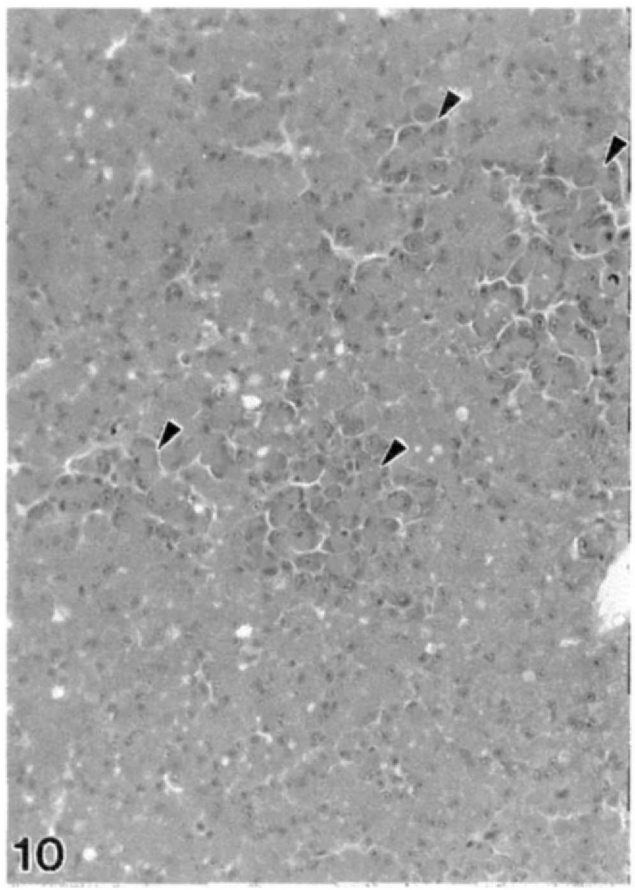
FIG. 9.—Adenoma with area (V) containing highly vacuolated cells with reduced amounts of zymogen (arrowheads) and loss of tubular structure. H&E. ×614.

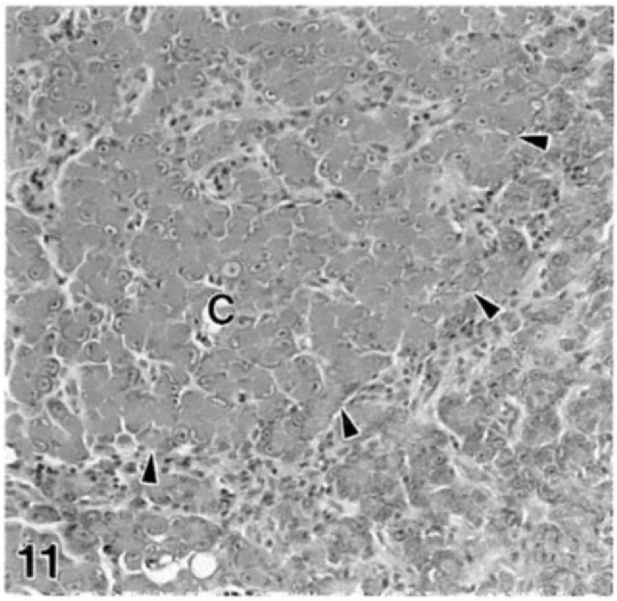
FIG. 10.—Adenoma exhibiting foci of atypical acinar cells (arrowheads). H&E. ×244.

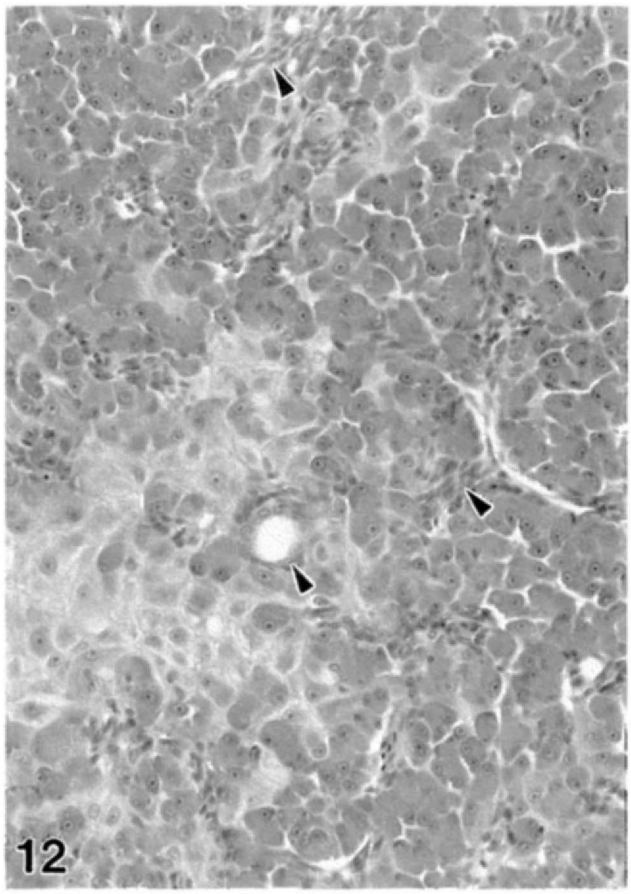


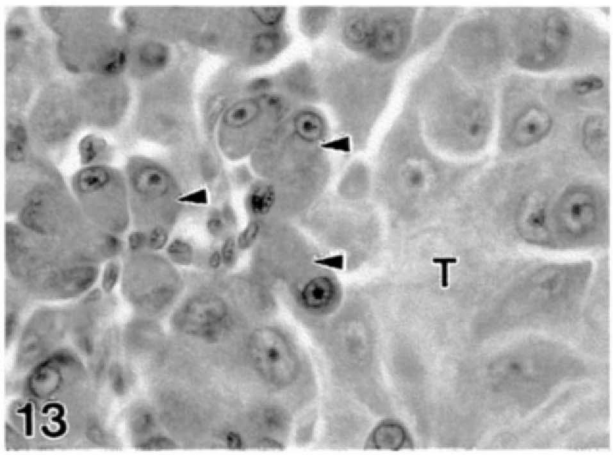


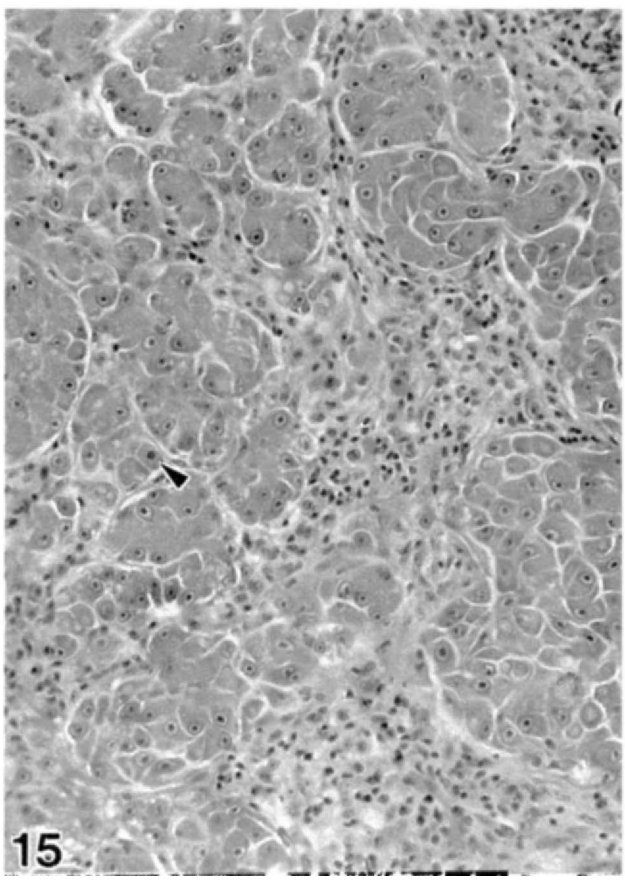


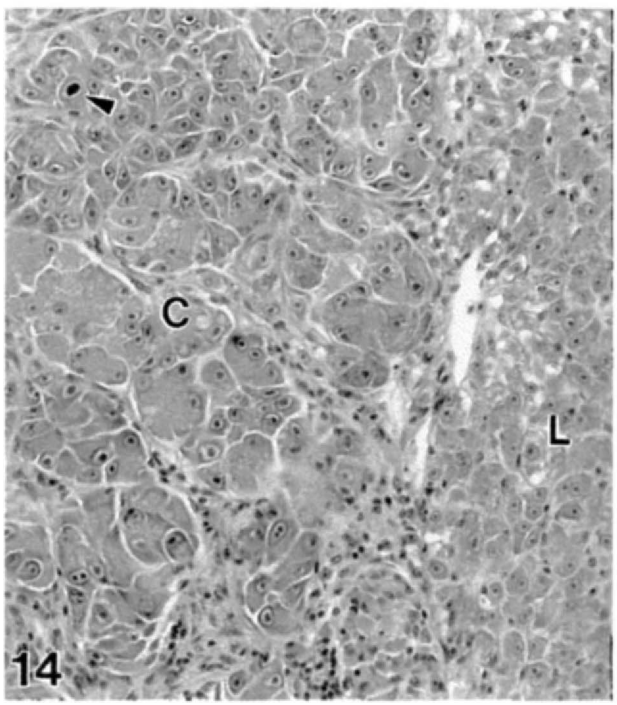












tissue surrounding the gallbladder of 1 specimen, and in the intrahepatic pancreas of 1 specimen. Grossly visible lesions were creamy white in color, smooth, and ranged in size and shape from 1.0 mm spherical masses to 5.0 mm multinodular masses (Fig. 1). Microscopically, adenomas usually were composed of well-differentiated acinar cells organized into branching and anastomosing tubules (Figs. 5–8). Acinar cells had basally located, spherical nuclei and numerous eosinophilic zymogen granules in the apical cytoplasm (Fig. 6). Some nuclei were enlarged with prominent nucleoli. Mitotic figures were present but occurred in low numbers. A prominent stromal component occurred among pancreatic tubules in some of the lesions (Fig. 6). Profiles of pancreatic ducts were rare or absent, even in the larger neoplasms.

Two large adenomas, one of which arose in the liver (Fig. 7), contained areas showing a progressive loss of typical architectural features and an increase in cellular atypia. The majority of acinar cell nuclei from 1 case exhibited severe nuclear pleomorphism (Fig. 8). Nuclei were greatly elongated, flattened, and displaced to the periphery of the cells. Another atypical adenoma contained basophilic areas exhibiting a disrupted architecture. Individual cells were highly vacuolated (Fig. 9), showed reduced contact with adjacent cells, contained a reduced amount or a total lack of zymogen, and contained single, highly basophilic bodies in the cytoplasm.

One large acinar cell adenoma contained small foci of more atypical cells (Fig. 10). The prominent tubular architecture was disrupted in these foci, with constituent acinar cells exhibiting loss of polarity, increased cytoplasmic basophilia, reduced zymogen content, and increased nuclear pleomorphism and mitotic activity.

Acinar Cell Carcinoma

Acinar cell carcinomas occurred in 9 specimens. Two cases developed in mesenteric pancreas and 7 cases in intrahepatic pancreatic tissue. The carcinomas were all locally invasive, and none exhibited areas of prominent ductal proliferation. One mesenteric acinar cell carcinoma was a small lesion that apparently arose within normal exocrine pancreas in the hilar region of the liver. The normal architecture was disrupted and the neoplastic cells showed a reduced basal basophilia, extensive pleomorphism, and nuclear atypia (Fig. 11). Many nuclei were enlarged with prominent nucleoli, and many were crescent-shaped and compressed at the periphery of the acinar cells. Abnormal mitotic figures were also present. The second mesenteric tumor was a creamy white, multilobulated mass that measured 11.0 × 5.0 mm (Fig. 2). Histologically, the acinar cells of this lesion exhibited a wide range of differentiation (Fig. 12). Well-differentiated areas had acinar cells organized as tubules, with full complements of zymogen granules, characteristic basal basophilia, and spherical nuclei with central nucleoli. Poorly differentiated areas showed almost a complete loss of tubular architecture and contained some cells totally devoid of zymogen (Fig. 13). Cellular pleomorphism and nuclear atypia were more evident in the less well differentiated areas; however, mitotic figures were abundant throughout the tumor mass.

Intrahepatic acinar cell carcinomas were large, aggressive lesions that ranged in size from approximately 2.0 to 8.0 mm in greatest dimension. Several of these neoplasms infiltrated and replaced large portions of the hepatic parenchyma (Fig. 14). Neoplastic cells in some lesions were arranged in distinct lobules separated by variable amounts of stroma (Fig. 15). Tumor cells were irregularly shaped and had a basophilic cytoplasm, and some were multinucleate. Irregularly shaped nuclei containing multiple nucleoli were common features in these lesions. Both normal and abnormal mitotic figures were common in these tumors. Some areas in each carcinoma examined had normal-appearing acinar cells with full complements of zymogen granules (Fig. 12).

←

FIG. 11.—Small acinar cell carcinoma (C) comprised of poorly differentiated acinar cells showing cellular pleomorphism and nuclear atypia. Arrowheads indicate border of neoplasm. H&E. ×244.

FIG. 12.—Light micrograph of carcinoma illustrated in Fig. 2. Note both well-differentiated cells containing zymogen and paler, more poorly differentiated cells lacking zymogen granules. Also note proliferating ductular elements (arrowheads). H&E. ×244.

FIG. 13.—High magnification of same neoplasm illustrated in Fig. 12. Note well-differentiated acinar cells containing abundant zymogen (arrowheads) and less well differentiated tumor cells lacking zymogen (T). H&E. ×614.

FIG. 14.—Intrahepatic acinar cell carcinoma (C) showing extensive cellular pleomorphism, nuclear atypia, and border with hepatic parenchyma (L). Arrowhead indicates mitotic figure. H&E. ×244.

FIG. 15.—Same tumor illustrated in Fig. 14 showing lobular architecture, prominent stroma (S), and leukocytic infiltration. Arrowhead indicates mitotic figure. H&E. ×244.

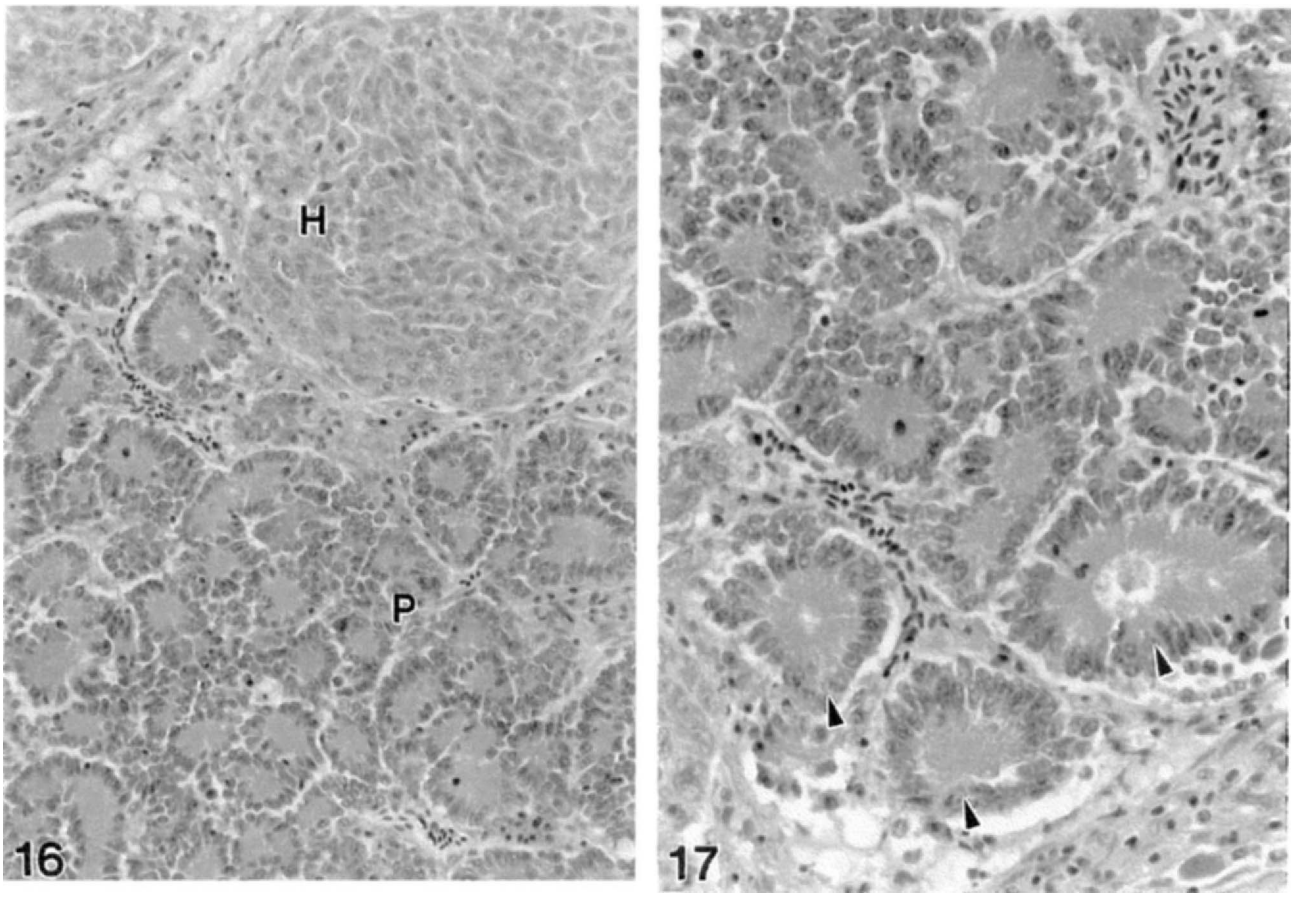


FIG. 16.—Intrahepatic acinar cell carcinoma (P) with tubular pattern arising within an hepatocellular carcinoma (H). H&E. ×244.

FIG. 17.—Higher magnification of Fig. 16 showing distinct tubules (arrowheads) with peripherally situated nuclei and central lumina. H&E. ×390.

One intrahepatic acinar cell carcinoma exhibited a prominent tubular or glandular pattern (Fig. 16) and apparently arose within a poorly differentiated hepatocellular carcinoma. The pancreatic portion of this tumor was well differentiated with neoplastic cells forming distinct tubular profiles, many containing variably shaped lumina with internal limiting membranes. Tubule cells were elongated and contained numerous small, refractile eosinophilic granules. Nuclei were peripherally located, highly basophilic, and somewhat elongated (Fig. 17). Normal and abnormal mitotic figures were abundant throughout the lesion. Ultrastructural examination of tissue reprocessed from the paraffin block confirmed the acinar cell origin of this neoplasm. Tumor cells contained abundant rough endoplasmic reticulum and numerous small zymogen granules.

DISCUSSION

Most other exocrine pancreatic neoplasms observed in wild fishes occurred as isolated cases in diverse species (8) and apparently were not clearly linked with environmental contamination. These reports included acinar cell adenomas in a European flounder and a greater redhorse (7); purported ductal adenomas in an oyster toadfish (31); pancreatic carcinomas from a goldfish (29), an Atlantic tomcod (12), and a coho salmon (17); and an acinar cell adenocarcinoma in a hybrid platyfish (28). This is the first report of multiple cases of exocrine pancreatic neoplasia in a wild fish population inhabiting a chemically contaminated environment. The lesions ranged from a single small focal lesion resembling an acidophilic focus to small, benign-appearing acinar cell adenomas to large, aggressive acinar cell carcinomas. The occurrence of exocrine pancreatic neoplasia in mummichog constitutes an epizootic as defined by Harshbarger and Clark (13), who considered an epizootic as at least 3 cases, in a single species, of neoplasms originating from a specific cell lineage from a discrete geographic location. In this report, we described 20 cases of exocrine pancreatic neoplasms in mummichog inhab-

iting a small tidal creek adjacent to a wood-treatment facility on the Elizabeth River, Virginia. In addition to the pancreatic neoplasms, this fish population also exhibited a broad spectrum of liver lesions (33) and elevated prevalences of extrahepatic proliferative lesions including neoplasms of the bile ducts, vascular system, kidney, and lymphoid tissues (Vogelbein, unpublished observations). To date, neoplasms have been confirmed in 5 different organ systems arising from 8 cell types in mummichog from this site. These findings and the extremely high PAH concentrations in sediment at this locality (33) strongly suggest a chemical etiology for these lesions.

Classification of the mummichog exocrine pancreatic neoplasms was based on morphological criteria of exocrine pancreatic neoplasms of mammals (3, 20) and fish (8, 9). These fish classification schemes were based on the light microscopic cytomorphologic features and biologic behavior of the neoplasms. Acinar cell adenomas were generally well-differentiated lesions characterized by a substantial increase in exocrine pancreatic tissue organized into well-defined, irregularly shaped masses. Larger lesions showed a loss of characteristic architectural features and an increase in cellular pleomorphism. Carcinomas usually contained some well-differentiated acinar cells. Neoplastic cells were arranged in tubular or lobular patterns and, in some areas, formed solid sheets. Carcinomas exhibited extensive cellular pleomorphism and nuclear atypia. Both normal and abnormal mitoses were abundant, and the lesions were locally invasive.

Fournie et al (9) reported atypical acinar cell foci within adenomas of 2 guppies exposed to methylazoxymethanol acetate. These foci consisted of acinar cells with basophilic, fibrillar cytoplasm devoid of zymogen granules and were considered to indicate progression toward carcinoma. At least 1 mummichog adenoma exhibited similar atypical acinar cell foci. However, the small focal lesion arising in normal mesenteric pancreas of another specimen more closely resembled the acidophilic focus reported in rats exposed to azaserine (21) and 4-hydroxyaminoquinoline-1-oxide (30). This acidophilic focus was considered to be the probable precursor of acinar cell adenomas and carcinomas in these rodent models and may represent a similar preneoplastic stage in the mummichog as well.

A chemical etiology for the mummichog pancreatic neoplasms is suggested by the fact that exocrine pancreatic neoplasms have been experimentally induced in several small fish species or have occurred in a large group of fishes used in a variety of carcinogenicity tests (15). Mummichog exocrine pancreatic neoplasms displayed several morphological features similar to those of other small fish species. Mummichog acinar cell adenomas and carcinomas resembled those induced in the guppy, *Poecilia reticulata,* by exposure to methylazoxymethanol acetate, a direct-acting carcinogen (9) in both their behavior and histology. Both species developed tumors that were large compared to the size of the specimens, and the cellular patterns of the lesions were similar. The mummichog acinar cell carcinoma exhibiting the prominent tubular pattern also resembled an acinar cell carcinoma described from a gulf killifish, *Fundulus grandis,* exposed to the highly mutagenic and carcinogenic compound *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (11). Neoplastic cells in both tumors formed distinct tubular profiles and arose from intrahepatic exocrine pancreatic tissue. Although the medaka pancreatic carcinomas (15) occurred only in the mesenteric pancreas and were more poorly differentiated than the mummichog neoplasms, the aggressive nature of the tumors in both species was evidenced by their size, elevated mitotic activity, and growth patterns. In contrast, the acinar cell neoplasms in mummichog bore no resemblance to the purported pancreatic neoplasms of duct cell origin induced by diethylnitrosamine in the mangrove rivulus, *Rivulus marmoratus* (32).

Mummichog exocrine pancreatic neoplasms were also similar to those in mammals, particularly the Fischer-344 and LEW rats (3, 4, 6, 22, 23). Acinar cell adenomas and carcinomas are the most commonly occurring types of neoplasms in both species, and the histologic patterns exhibited by the lesions are strikingly similar. Adenomas in the rat strains were well-differentiated lesions that showed loss of normal architectural features, increased cellular pleomorphism, and a tendency to organize into branching tubules. Pancreatic carcinomas displayed a range of differentiation but clearly originated from exocrine pancreatic acinar cells. Thus, pancreatic neoplasms in both rats and fishes are believed to be of acinar cell origin, a hypothesis that is supported by the histologic type of the neoplasms and the absence of hyperplastic changes in ductal epithelium.

The mummichog is a small, shallow-water cyprinodontid fish that exhibits a restricted home range (10, 24). Although local populations are nonmigratory, the species has a broad geographic distribution, inhabiting tidal marshes from Nova Scotia to Florida (2). Local subpopulations may therefore reflect the condition of their immediate environment. Because of the mummichog's biology and apparent tumorigenic sensitivity, developing hepatic (33) and extrahepatic neoplasms, this fish may serve as a sentinel species for chemical carcinogens in coastal waters along the eastern seaboard of the United States.

ACKNOWLEDGMENTS

This study was supported in part by U.S. Environmental Protection Agency Cooperative Agreement CR 818165-01-0. We wish to thank Pat Blake for the preparation of histologic slides, Patrice Mason for reprocessing paraffin-embedded tissue for electron microscopy, and Valerie Coseo for typing the manuscript. We gratefully acknowledge Dr. William E. Hawkins for reviewing the manuscript. This paper is contribution number 1856 of the Virginia Institute of Marine Science and number 858 of the U.S. EPA, Environmental Research Laboratory, Gulf Breeze, Florida.

REFERENCES

1. Baumann PC (1989). PAH, metabolites, and neoplasia in feral fish populations. In: *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment,* U Varanasi (ed). CRC Press, Boca Raton, Florida, pp. 269–289.
2. Bigelow HB and Schroeder WC (1953). Fishes of the Gulf of Maine. *U.S. Fish Wildl. Serv. Fish Bull.* 53: 1–77.
3. Boorman GA and Eustis SL (1984). Proliferative lesions of the exocrine pancreas in male F344/N rats. *Environ. Health Persp.* 71: 5–16.
4. Boorman GA and Eustis SL (1990). Neoplasms of the exocrine pancreas. In: *Atlas of Tumor Pathology of the Fischer Rat,* SF Stinson, HM Schuller, and GK Reznik (eds). CRC Press, Boca Raton, Florida, pp. 221–225.
5. Couch JA and Harshbarger JC (1985). Effects of carcinogenic agents on aquatic animals: An environmental and experimental overview. *Environ. Carcinog. Rev.* 3: 63–105.
6. Eustis SL and Boorman GA (1985). Proliferative lesions of the exocrine pancreas: Relationship to corn oil gavage in the National Toxicology Program. *J. Natl. Cancer Inst.* 75: 1067–1073.
7. Fournie JW, Black JJ, and Vethaak AD (1988). Exocrine pancreatic adenomas in the greater redhorse, *Moxostoma valenciennesi* Jordan, and in the European flounder, *Platichthys flesus* (L). *J. Fish Dis.* 11: 445–448.
8. Fournie JW and Hawkins WE (in press). Neoplasms of the exocrine pancreas. In: *Pathology of Spontaneous and Induced Neoplasms in Fishes: Comparative Characterization, Nomenclature, and Literature,* CJ Dawe, JC Harshbarger, SR Wellings, and JD Strandberg (eds). Academic Press, San Diego.
9. Fournie JW, Hawkins WE, Overstreet RM, and Walker WW (1987). Exocrine pancreatic neoplasms induced by methylazoxymethanol acetate in the guppy (*Poecilia reticulata*). *J. Natl. Cancer Inst.* 78: 715–725.
10. Fritz ES, Meredith WH, and Lotrich VA (1975). Fall and winter movements and activity level of the mummichog, *Fundulus heteroclitus,* in a tidal creek. *Chesapeake Sci.* 16: 211–215.
11. Grizzle JM, Putnam MR, Fournie JW, and Couch JA (1988). Microinjection of chemical carcinogens into small fish embryos: Exocrine pancreatic neoplasm in *Fundulus grandis* exposed to N-methyl-N'-nitro-N-nitrosoguanidine. *Dis. Aquat. Org.* 5: 101–105.
12. Harshbarger JC (1965–1981). *Activities Reports.* Registry of Tumors in Lower Animals, Smithsonian Institution, Washington, D.C.
13. Harshbarger JC and Clark JB (1990). Epizootiology of neoplasms in bony fish of North America. *Sci. Total Environ.* 94: 1–32.
14. Harshbarger JC, Spero PM, and Wolcott NM (1992). Neoplasms in wild fish from the marine ecosystem emphasizing environmental interactions. In: *Pathobiology of Marine and Estuarine Organisms,* JA Couch and JW Fournie (eds). CRC Press, Boca Raton, Florida, pp. 157–176.
15. Hawkins WE, Fournie JW, Battalora MJ, and Walker WW (1991). Carcinoma of the exocrine pancreas in medaka (*Oryzias latipes*). *J. Aquat. Anim. Health* 3: 213–220.
16. Hayat MA (1989). *Principles and Techniques of Electron Microscopy,* 3rd ed. CRC Press, Boca Raton, Florida, p. 469.
17. Heidel JR, Rockey DD, and Duimstra JR (1992). Acinar cell carcinoma of the pancreas in coho salmon *Oncorhynchus kisutch. Dis. Aquat. Org.* 13: 151–154.
18. Hendricks JD (1982). Chemical carcinogenesis in fish. In: *Aquatic Toxicology,* LJ Weber (ed). Raven Press, New York, pp. 149–211.
19. Hoover KL (1984). *National Cancer Institute Monograph 65: Use of Small Fish Species in Carcinogenicity Testing.* NIH Publication No. 84-2653, National Institutes of Health, Bethesda, Maryland.
20. Lack EE (1989). Primary tumors of the exocrine pancreas. *Am. J. Surg. Pathol.* 13(suppl. 1): 66–88.
21. Longnecker DS (1987). The azaserine-induced model of pancreatic carcinogenesis in rats. In: *Experimental Pancreatic Carcinogenesis,* JK Reddy and DS Longnecker (eds). CRC Press, Boca Raton, Florida, pp. 117–130.
22. Longnecker DS, Roebuck BD, Kuhlman ET, and Curphey TJ (1985). Induction of pancreatic carcinomas in rats with N-nitroso(2-hydroxypropyl) (2-oxopropyl)amine: Histopathology. *J. Natl. Cancer Inst.* 74: 209–217.
23. Longnecker DS, Roebuck BD, Yager JD, Lilja HS, and Siegmund B (1981). Pancreatic carcinoma in azaserine-treated rats: Induction, classification and dietary modulation of incidence. *Cancer* 47: 1562–1572.
24. Lotrich VA (1975). Summer home range and movements of *Fundulus heteroclitus* (Pisces: Cyprinodontidae) in a tidal creek. *Ecology* 56: 191–198.
25. Luna LG (1968). *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology,* 3rd ed. McGraw-Hill, New York, p. 258.
26. Malins DC, Krahn MM, Myers MS, Rhodes LD, Brown DW, Krone CA, McCain BB, and Chan S (1985). Toxic chemicals in sediments and biota from a creosote-polluted harbor: Relationships with he-

patic neoplasms and other hepatic lesions in English sole (*Parophrys vetulus*). *Carcinogenesis* 6: 1463–1469.
27. Malins DC, McCain BB, Myers MS, Brown DW, Krahn MM, Roubal WT, Schiewe MH, Landahl JT, and Chan SL (1987). Field and laboratory studies of the etiology of liver neoplasms in marine fish from Puget Sound. *Environ. Health Persp.* 71: 5–16.
28. Nigrelli RF and Gordon M (1951). Spontaneous neoplasms in fishes. V. Acinar adenocarcinoma of the pancreas in a hybrid platyfish. *Zoologica* 36: 121–126.
29. Otte E (1964). Eine bösartige Neubildung in der Bauchhöhle eines Goldfisches (*Carassius auratus* L.). *Wein Tieraerztl. Monatsschr.* 51: 485–488.
30. Rao MS, Subbarao V, and Scarpelli DG (1987). 4-Hydroxy-aminoquinoline-1-oxide-induced acinar cell lesions of the rat pancreas. In: *Experimental Pancreatic Carcinogenesis,* DG Scarpelli, JK Reddy, and DS Longnecker (eds). CRC Press, Boca Raton, Florida, pp. 107–116.
31. Thiyagarajah A and Bender MD (1988). Lesions in the pancreas and liver of an oyster toadfish, *Opsanus tau* (L.), collected from the lower York River, Virginia, USA. *J. Fish Dis.* 11: 359–364.
32. Thiyagarajah A and Grizzle JM (1986). Diethylnitrosamine-induced pancreatic neoplasms in the fish *Rivulus ocellatus marmoratus. J. Natl. Cancer Inst.* 77: 141–147.
33. Vogelbein WK, Fournie JW, Van Veld PA, and Huggett RJ. (1990). Hepatic neoplasms in the mummichog *Fundulus heteroclitus* from a creosote-contaminated site. *Cancer Res.* 50: 5978–5986.